



Original communication

Evaluation of the morphological changes of gastric mucosa induced by a low concentration of acetic acid using a rat model



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ABSTRACT

Oral ingestion of concentrated acetic acid causes corrosive injury of the gastrointestinal tract. To assess the effects of a low concentration of acetic acid on gastric mucosa, we examined the gastric mucosal changes in rats at 1 and 3 days after the injection of 5% or 25% acetic acid into the gastric lumen. The area of the gastric ulcerative lesions in the 25% acetic acid group was significantly larger than that in the 5% acetic acid group. The lesion area was reduced significantly at 3 days after injection in the 5% acetic acid group, whereas no significant difference in lesion area was observed at 1 and 3 days in the 25% acetic acid group. Histologically, corrosive necrosis was limited to the mucosal layer in the 5% acetic acid group, whereas necrosis extended throughout the gastric wall in the 25% acetic acid group. At 3 days post-injection, the 25% acetic acid group showed widespread persistent inflammation, whereas the 5% acetic acid group showed widespread appearance of fibroblasts indicative of a healing process. These results indicate that a low concentration of acetic acid damages the gastric mucosa and that the degree of mucosal damage depends on the concentration of acetic acid.

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1. Introduction

Oral ingestion of concentrated acetic acid causes corrosive injury of the oropharyngeal mucosa, erosion of esophageal and gastric mucosa, and perforation of the stomach.¹ Systemic absorption of acetic acid induces hemolysis, acute renal failure, acute liver failure, disseminated intravascular coagulation, and circulatory shock.^{2,3} Previous reports have described corrosive injury of the upper alimentary tract due to accidental or suicidal ingestion of 30–96% acetic acid.^{1–5} However, information on the effects of low concentration acetic acid on the intestinal mucosa is scarce. Only two cases have been reported on corrosive esophagitis or gastroenteritis following ingestion of vinegar, a popular flavoring agent in cooking, containing 4–5% acetic acid.^{6,7} In addition, 1–4% acetic acid is used for diagnostic and sterilization purposes in medical practice.^{8–10} Recently, news media have reported two fatal cases of intestinal necrosis after ingestion of incorrectly diluted 25% acetic acid at gastroscopy or sterilization of gastrostomy tube in hospitals.^{11,12} Information of the effects of low concentration acetic acid

on the intestinal mucosa is essential in forensic autopsy of the victims to prove a causal connection between medical malpractice and death. It is therefore important to elucidate the changes that are induced in the intestinal mucosa by low concentrations of acetic acid by using an animal model. In the present study, we used acetic acid concentrations of 5% and 25% to induce gastric mucosal lesions in rats and analyzed the resulting macroscopic and histological changes to the gastric mucosa over 3 days to assess the impact of gastric acetic acid concentrations on mucosal damage.

2. Materials and methods

2.1. Animals

The Ethical Review Committee of Animal Experiments at Tokyo Women's Medical University approved the animal protocol. Six-week-old male Wistar rats weighing between 160 and 200 g were used. Rats were housed in groups of 3 per cage and acclimatized to standard laboratory conditions (light–dark cycle, 12 h/12 h; temperature, 23 °C; and free access to food and water) for 1 week before the experiment. The animals were kept in cages with raised mesh bottoms to prevent coprophagy, and were deprived of food but allowed free access to tap water for 24 h before the experiment.

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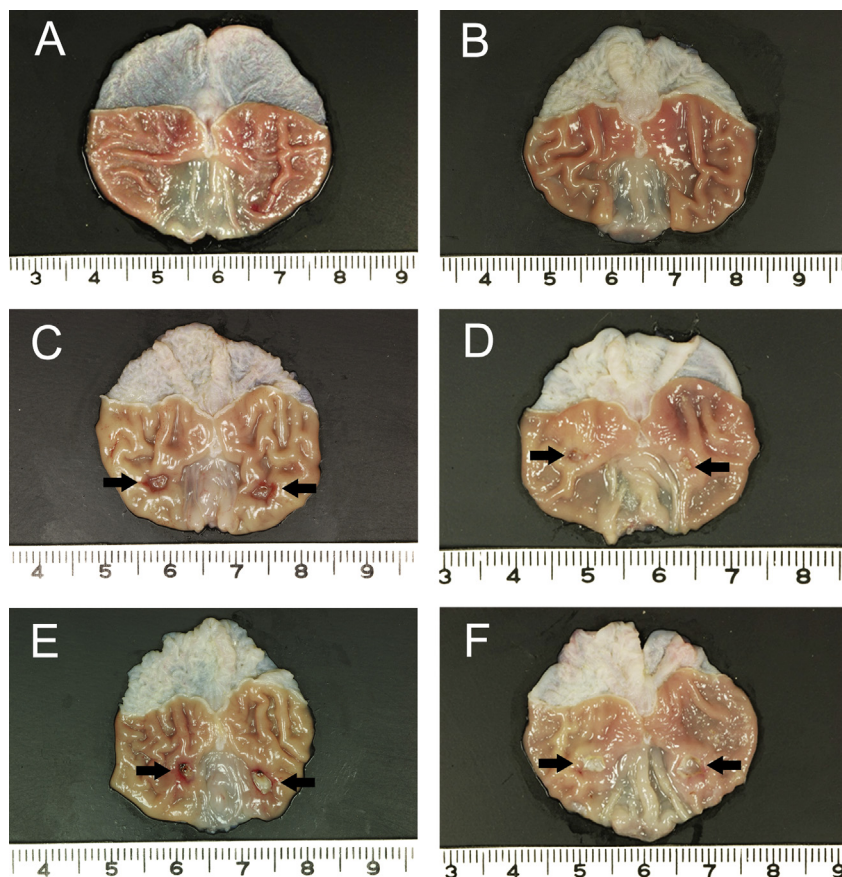


Fig. 1. Macroscopic appearance of the gastric mucosal surface. (A, B) After the injection of saline. (C, D) After the injection of 5% acetic acid. (E, F) After the injection of 25% acetic acid. (A, C, E) 1 day after injection. (B, D, F) 3 days after injection. Arrows indicate ulcerative lesions on the mucosa of the anterior and posterior gastric walls.

2.2. Surgical preparation

Gastric mucosal lesions were induced by acetic acid solution using Tsukimi and Okabe's method with modifications. This method produces an ulcerative lesion on the mucosa of both the anterior and posterior gastric walls.¹³ Solutions of 5% or 25% acetic acid in saline were made using glacial acetic acid (Alfa Aesar, Ward Hill, Massachusetts, USA). The rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg) and their abdomens were surgically opened. The stomach was exposed and clamped at the center of the corpus with ring forceps with an inner diameter of 5 mm. A 26-G needle was inserted into the gastric lumen between the anterior and posterior gastric walls at the clamped portion. Thereafter, 50 μ L of 5% or 25% acetic acid was injected into the gastric lumen through the needle. At 40 s following the injection, the acetic acid solution was aspirated from the gastric lumen. The abdomen was closed using sutures. Control rats were subjected to the same surgical procedure, but received 50 μ L of saline instead of acetic acid solution. The animals were returned to the cages and were allowed free access to food and water until use.

2.3. Measurement of the ulcer area

The rats were killed by general anesthesia with an intraperitoneal injection of pentobarbital (100 mg/kg) at 1 or 3 days after the injection of 5% acetic acid, 25% acetic acid, or saline ($n = 3$ per group and 18 in total). Stomachs were removed, opened along the greater curvature, and thoroughly rinsed with saline. The mucosal surface of the stomach was photographed using a digital camera. The areas

of the gastric mucosal lesions that were defined as a defect of gastric mucosa on the anterior and posterior gastric walls were manually traced and measured using Image J software (ver. 1.44). The sum of the area of both lesions was expressed as the "lesion size" for each animal.

2.4. Histological procedures

Tissue sections of the injection site of the gastric wall were taken from the stomach and fixed in a 20% phosphate buffered

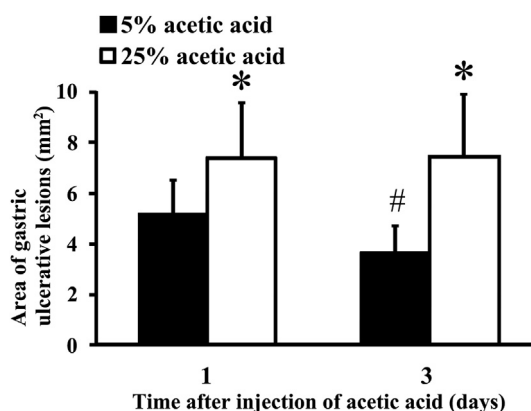


Fig. 2. Area of the gastric mucosal lesions at 1 and 3 days after the injection of acetic acid. Values are expressed as mean \pm standard deviation. $N = 3$ in each group. *Significant difference between the 5% and 25% acetic acid groups at $p < 0.05$. # Significant difference between 1 day and 3 days after injection at $p < 0.05$.

formalin solution for 24 h. The sections were dehydrated through a graded series of alcohol solutions, cleared with xylene, and embedded in paraffin. Serial sections of 4- μ m thickness were cut from the paraffin blocks and mounted on slides. Prior to staining, sections mounted on slides were dewaxed and hydrated through a graded ethanol series. Hematoxylin and eosin (H&E) staining, High Iron Diamine-Alcian blue pH 2.5 staining for small vessels, Masson's trichrome staining for fibroblasts were performed on the tissue sections. Neutrophils were detected using a naphthol AS-D chloroacetate staining kit according to the manufacturer's protocols (Muto Pure Chemicals Ltd., Tokyo, Japan). The numbers of neutrophils and fibroblasts in 1 mm² of submucosal layer at the margin of the ulcerative lesion were counted under microscopy.¹⁴ We compared histological findings among the saline, 5% acetic acid, and 25% acetic acid groups at 1 and 3 days post-injection.

2.5. Statistics

The area of gastric mucosal lesions and the number of neutrophils and fibroblasts in the submucosal layer were expressed as mean \pm standard deviation (SD) in each experimental group. Between-group differences were determined using Student's *t*-tests, with *p* < 0.05 considered statistically significant.

3. Results

3.1. Macroscopic observations

In the saline group, gastric mucosal lesions were not observed at 1 and 3 days post-injection. In the 5% and 25% acetic acid groups, ulcerative lesions with a round or oval shape, and clear and reddish

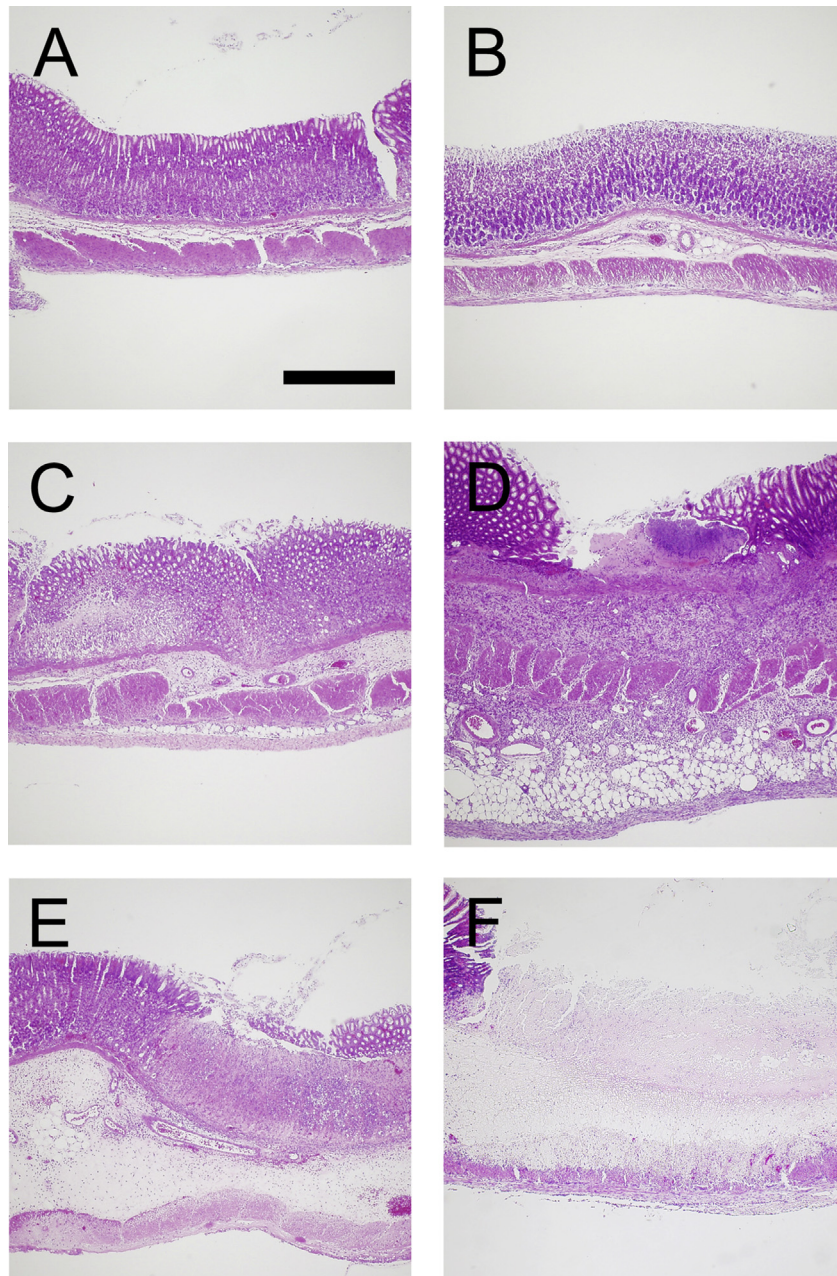


Fig. 3. Histology sections of the gastric wall (hematoxylin and eosin staining). (A, B) After the injection of saline. (C, D) After the injection of 5% acetic acid. (E, F) After the injection of 25% acetic acid. (A, C, E) 1 day after injection. (B, D, F) 3 days after injection. Scale bar = 500 μ m.

margins, were observed on the anterior and posterior walls of the stomach at 1 and 3 days following injection (Fig. 1).

3.2. Area of the gastric ulcerative lesions

The area of the gastric ulcerative lesions produced by 25% acetic acid was significantly larger than the area of the lesions produced by 5% acetic acid at 1 and 3 days following injection ($p < 0.05$). In the 5% acetic acid group, the area of the gastric ulcerative lesions at 3 days post-injection was significantly reduced compared to the lesion area observed 1 day post-injection ($p < 0.05$). In the 25% acetic acid group, no significant differences in the area of gastric ulcerative lesions were observed between days 1 and 3 following injection ($p = 0.49$) (Fig. 2).

3.3. Histological findings

3.3.1. Depth of corrosive injury in gastric wall

Corrosive injury was not observed in the saline group (Fig. 3A and B). In the 5% acetic acid group, necrosis was observed in the mucosal layer at both 1 and 3 days following injection (Fig. 3C and D). In the 25% acetic acid group, necrosis extended from the mucosal layer through the submucosal and muscular layers to the serosa at 1 and 3 days after injection (Fig. 3E and F).

3.3.2. Small blood vessel damages in submucosal layer

In the saline and 5% acetic acid groups, small vessels in submucosal layer showed no specific changes at 1 and 3 days after injection (Fig. 4A–D). In the 25% acetic acid group, coagulation

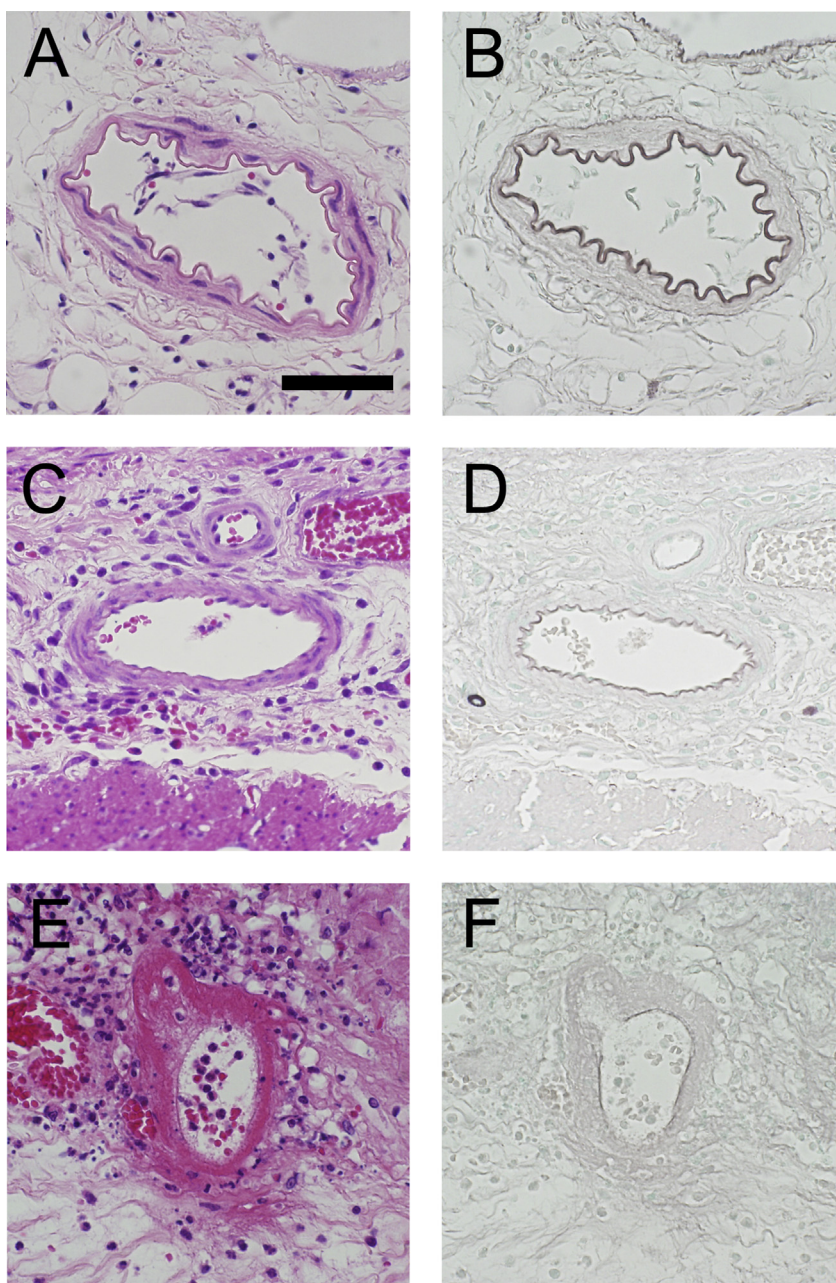


Fig. 4. Blood vessels in the submucosal layer of the gastric wall. (A, C, E) Hematoxylin and eosin staining. (B, D, F) High Iron Diamine-Alcian blue pH 2.5 staining. (A, B) After the injection of saline. (C, D) After the injection of 5% acetic acid. (E, F) After the injection of 25% acetic acid. Images A–F were obtained at 1 day after injection. Images obtained at 3 days after injection showed similar findings (data not shown). Scale bar = 50 μ m.

necrosis of small blood vessels was observed on H&E staining at 1 and 3 days after injection (Fig. 4E). High Iron Diamine-Alcian blue pH 2.5 staining revealed disruption of internal elastic lamina in the affected blood vessels (Fig. 4F).

3.3.3. Acute inflammatory response

In the saline group, a small number of neutrophils were observed in the mucosal layer at 1 day following injection; they were not found at 3 days post-injection (Fig. 5A and B). In the 5% acetic acid group, neutrophil infiltration extended from the mucosal layer through the submucosal and muscular layers to the serosa at 1 and 3 days post-injection (Fig. 5C and D). In the 25% acetic acid group, a large number of neutrophils infiltrated in all layers of the stomach wall at 1 and 3 days following injection

(Fig. 5E and F). The number of neutrophils in the submucosal layer in the 25% acetic acid group was significantly higher than that in the 5% acetic acid group at 1 and 3 days following injection ($p < 0.05$) (Fig. 6).

3.3.4. Healing process by fibroblasts infiltrations

In the 5% acetic acid groups, a large number of fibroblasts appeared in the mucosal and submucosal layers at 3 days following injection. In the 25% acetic acid group, a small number of fibroblasts were observed at the edge of the lesions at 3 days following injection (Fig. 7). In the 5% and 25% acetic acid groups, the number of the fibroblasts in the submucosal layer at 3 days post-injection was significantly increased compared to that at 1 day post-injection ($p < 0.01$). At 3 days after injection, the number

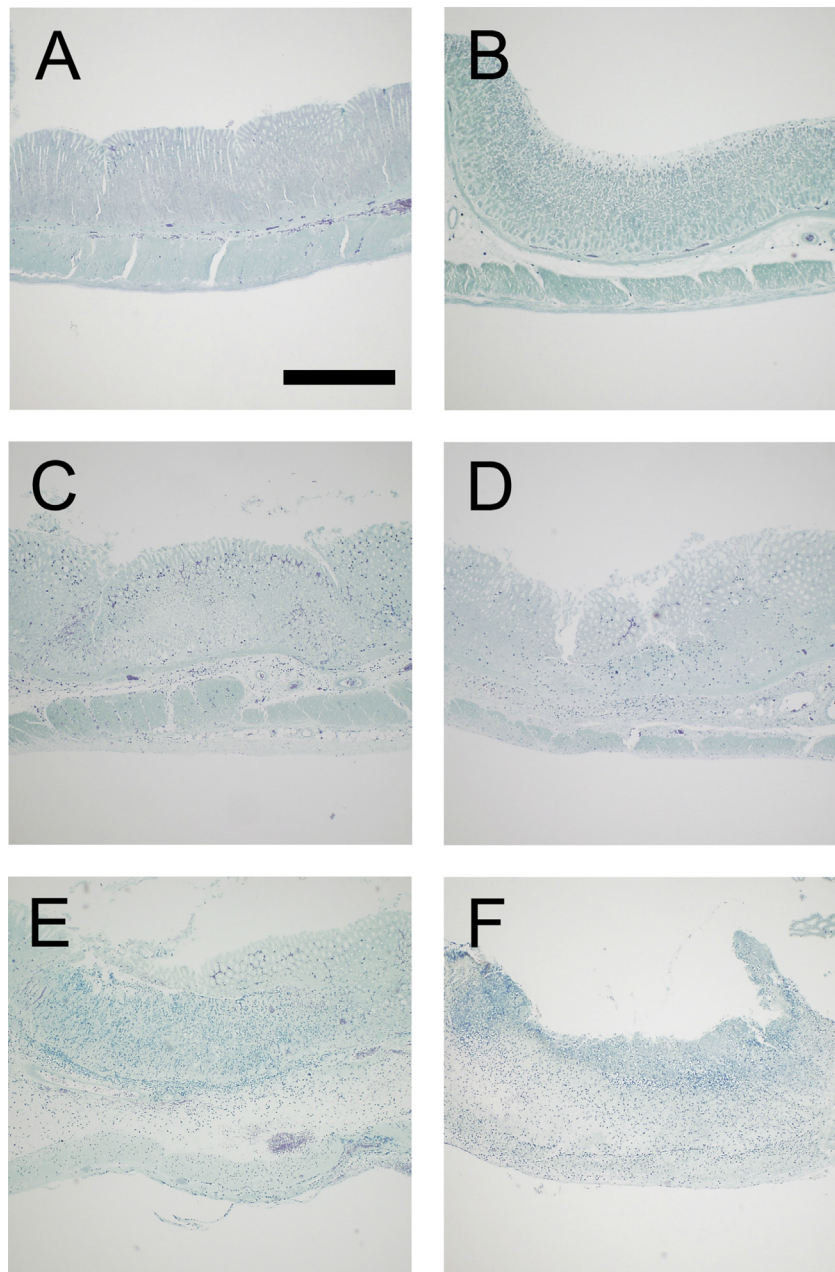


Fig. 5. Neutrophils in the gastric wall (naphthol AS-D chloroacetate staining). (A, B) After the injection of saline. (C, D) After the injection of 5% acetic acid. (E, F) After the injection of 25% acetic acid. (A, C, E) 1 day after injection. (B, D, F) 3 days after injection. Scale bar = 500 μ m.

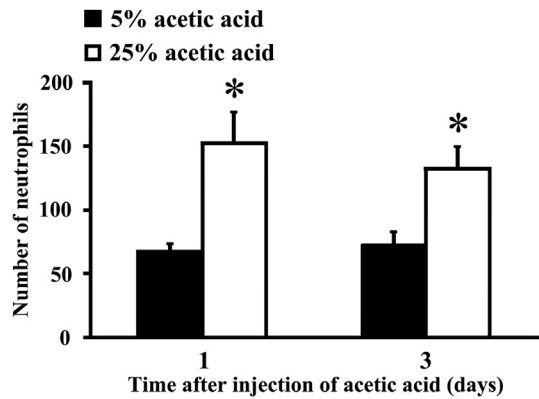


Fig. 6. Number of neutrophils in the gastric submucosal layer at 1 and 3 days after the injection of acetic acid. Values are expressed as mean \pm standard deviation. $N = 3$ in each group. *Significant difference between the 5% and 25% acetic acid groups at $p < 0.05$.

of the fibroblasts in the submucosal layer in the 25% acetic acid group was significantly lower than that in the 5% acetic acid group ($p < 0.01$) (Fig. 8).

4. Discussion

In the present study, we observed gastric mucosal changes at 1 and 3 days following the injection of 5% or 25% acetic acid into the gastric lumen in rats. Injections of acetic acid at concentrations of 5% as well as 25% successfully produced ulcerative lesions in the gastric mucosa. The area of the gastric ulcerative lesions in the 25% acetic acid group was significantly larger than in the 5% acetic acid group at 1 and 3 days post-injection. Histologically, corrosive necrosis was limited to the mucosal layer in the 5% acetic acid group, whereas necrosis extended from the mucosal layer through the submucosal and muscular layers to the serosa in the 25% acetic acid group at 1 and 3 days following injection. Moreover, coagulation

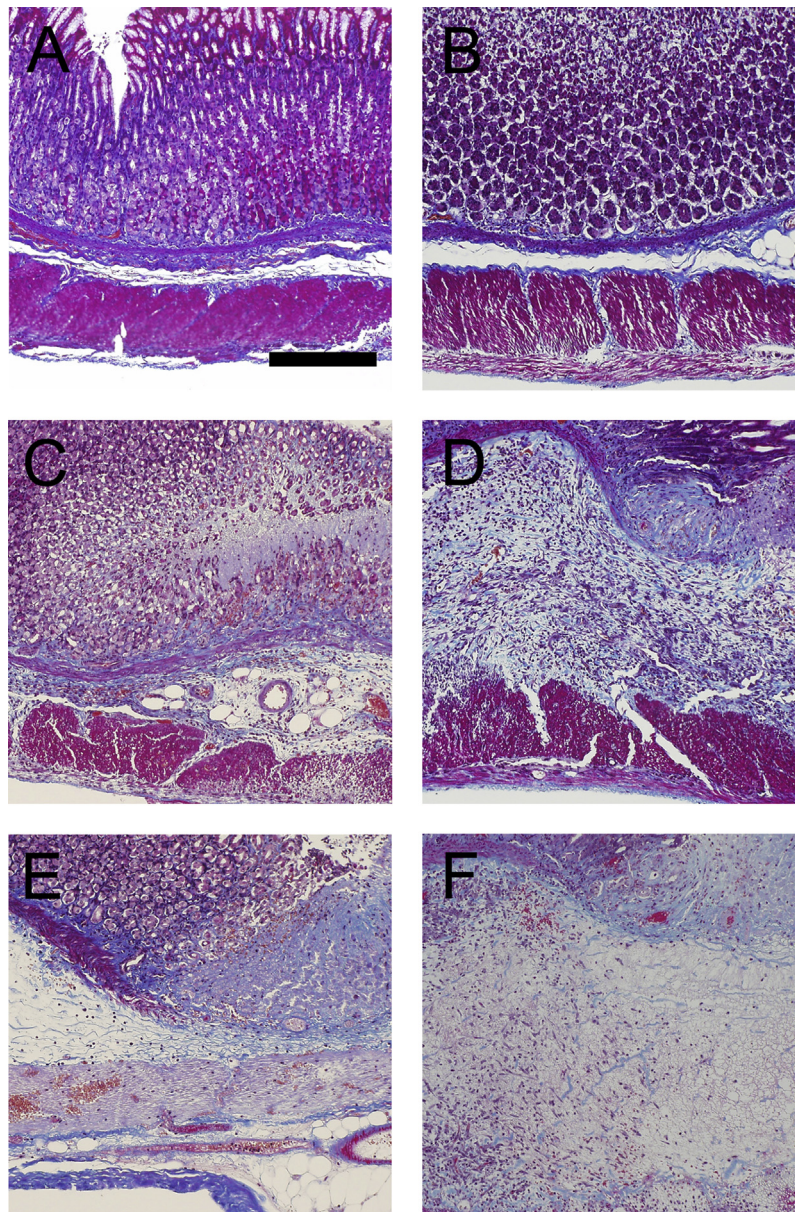


Fig. 7. Fibroblasts in the gastric wall (Masson's trichrome staining). (A, B) After the injection of saline. (C, D) After the injection of 5% acetic acid. (E, F) After the injection of 25% acetic acid. (A, C, E) 1 day after injection. (B, D, F) 3 days after injection. Scale bar = 200 μ m.

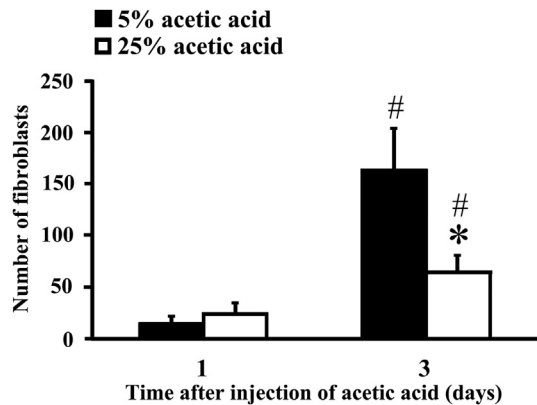


Fig. 8. Number of fibroblasts in the gastric submucosal layer at 1 and 3 days after the injection of acetic acid. Values are expressed as mean \pm standard deviation. $N = 3$ in each group. *Significant difference between the 5% and 25% acetic acid groups at $p < 0.05$. # Significant difference between 1 day and 3 days after injection at $p < 0.05$.

necrosis of small blood vessels with disruption of internal elastic lamina was observed in 25% acetic acid group. These results indicate that low concentrations of acetic acid damage the gastric mucosa and that the degree of damage depends on the concentration of the acetic acid.

Acetic acid has been used in experimental models to produce gastric mucosal lesions similar to human peptic ulcers. The animal model used in the present study is known as the “kissing ulcer” model, as it produces an ulcerative lesion on both the anterior and posterior walls and has been used to evaluate the effects of anti-ulcer drugs. According to the original method published by Tsukimi and Okabe,¹³ a ring forceps with a 9-mm inner diameter is used for clamping the stomach and 200 μ L of 60% acetic acid is injected into the gastric lumen for 60 s. In the present study, to examine the effect of a small volume and low concentration of acetic acid, a ring forceps with a 5-mm inner diameter was used for clamping, and 40 μ L of 5% or 25% acetic acid was injected into the gastric lumen for 40 s. We noted that even 5% acetic acid, the same concentration found in vinegar, damages the gastric mucosa in rats. Another experimental model has shown that the injection of 50 μ L of 1% acetic acid into the subserosal layer of the stomach produces ulcerative lesions in the gastric mucosa.¹⁵ Although the concentrations of acetic acid are 30–96% in the reported cases of corrosive injury due to acetic acid ingestion,^{1–5} our results suggest that 5–25% acetic acid also can cause corrosive injuries to the gastrointestinal tract in human.

In the present study, the gastric lesion area was significantly reduced at 3 days post-injection in the 5% acetic acid group, whereas there was no significant difference in lesion area between days 1 and 3 post-injection in the 25% acetic acid group. Histological examination revealed widespread inflammation at both 1 and 3 days following injection in the 25% acetic acid group, and the appearance of numerous fibroblasts at 3 days following injection in the 5% acetic acid group. The reduced area of the ulcerative lesions in association with the appearance of fibroblasts 3 days following injection in the 5% acetic acid group is indicative of a healing process. Tsukimi et al. reported that the area of the ulcerative lesions produced in the “kissing ulcer” model using 60% acetic acid is decreased at 7 days following injection, without the administration of antiulcer drugs.¹⁶ In the present study, if we had observed the rats for a longer time period, we may have observed a similar healing process in the ulcerative lesions produced by 25% acetic acid.

In hospital settings, 1.5–4% acetic acid is sprinkled on the gastric mucosa to visualize the lesion for biopsy at gastroscopy.^{8,9} Moreover, 1% acetic acid is injected into the catheter of percutaneous endoscopic gastrostomy patients to sterilize the lumen.¹⁰ Acetic acid solutions are also utilized in women undergoing colposcopy for the biopsy of uterine cervical cancers and in percutaneous injections for the treatment of hepatocellular carcinoma.^{9,17} These acetic acid solutions may be prepared from 100% acetic acid or vinegar solution by diluting the acetic acid with water. Our experimental study suggests that ingestion of 5–20% acetic acid causes intestinal necrosis in human. However, because acetic acid is classified as a weak acid and contained in kitchen vinegar, the adverse effects associated with this acid are not recognized. Hospital staff members should be made aware that low concentration acetic acid damages the mucosa, and should apply caution when handling and diluting acetic acid for use in patients.

Ethical approval

None declared.

Funding

None declared.

Conflict of interest

There is no conflict of interest.

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